Declaration

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Osaka, this 28th day of July , 2005

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[Title of the Invention] Chromatography Specimen, and Manufacturing Method thereof

[Claims]

[Claim 1] A chromatography specimen which is obtained by laminating plural porous materials or made of a single-layer porous material,

wherein a reactive layer on which at least one of reactive components adopted in a chromatographic analysis is immobilized includes a surface active agent having such a property that it can be solidified when dried.

[Claim 2] The chromatography specimen as defined in Claim 1,

wherein the surface active agent includes a surface active agent having a HLB value which is 20 or lower.

[Claim 3] The chromatography specimen as defined in Claim 1 or 2,

wherein the surface active agent includes a nonionic surface active agent.

[Claim 4] The chromatography specimen as defined in any of Claims 1 to 3,

wherein the surface active agent includes a cholic acid surface active agent.

[Claim 5] The chromatography specimen as defined in any of Claims 1 to 4,

wherein the surface active agent includes a surface

active agent having sugar in a hydrophilic part.

[Claim 6] The chromatography specimen as defined in any of Claims 1 to 5,

wherein the reactive layer includes the surface active agent in the entirety thereof.

[Claim 7] The chromatography specimen as defined in any of Claims 1 to 5,

wherein the reactive layer includes the surface active agent in a part thereof.

[Claim 8] A method for manufacturing a chromatography specimen which has a reactive layer on which at least one of reactive components adopted in a chromatographic analysis is immobilized comprising:

a step of impregnating or coating the reactive layer of the chromatography specimen with a surface active agent dissolved liquid in which a surface active agent having such a property that it can be solidified when dried is dissolved; and

a step of drying the surface active agent dissolved liquid with which the reactive layer has been impregnated or coated.

[Claim 9] The chromatography specimen manufacturing method as defined in Claim 8,

wherein the surface active agent includes a surface active agent having a HLB value which is 20 or lower.

[Claim 10] The chromatography specimen manufacturing method

as defined in Claim 8 or 9,

wherein the surface active agent includes a nonionic surface active agent.

[Claim 11] The chromatography specimen manufacturing method as defined in any of Claims 8 to 10,

wherein the surface active agent includes a cholic acid surface active agent.

[Claim 12] The chromatography specimen manufacturing method as defined in any of Claims 8 to 11,

wherein the surface active agent includes a surface active agent having sugar in a hydrophilic part.

[Claim 13] The chromatography specimen manufacturing method as defined in any of Claims 8 to 12,

wherein the reactive layer is dried by air drying.

[Claim 14] The chromatography specimen manufacturing method as defined in any of Claims 8 to 12,

wherein the reactive layer is dried by wind drying.

[Claim 15] The chromatography specimen manufacturing method as defined in any of Claims 8 to 12,

wherein the reactive layer is dried by freeze drying.

[Claim 16] The chromatography specimen manufacturing method as defined in any of Claims 8 to 15,

wherein the entire reactive layer is impregnated or coated with the surface active agent dissolved liquid.

[Claim 17] The chromatography specimen manufacturing method

as defined in any of Claims 8 to 15,
wherein a part of the reactive layer is impregnated or coated with the surface active agent dissolved liquid.

[Detailed Description of the Invention]

[0001]

[Technical Field of the Invention]

The present invention relates to a chromatography specimen for qualitatively or quantitatively analyzing a liquid sample and a manufacturing method thereof and, more particularly, to a specimen on which a liquid sample spreads uniformly.

[0002]

[Background Art]

Conventionally, a measuring method by chromatography, which utilizes an antigen-antibody reaction or enzyme reaction is generally used as a method for implementing a chemical test for liquid samples such as examination of water and urinalysis, or a clinical test. Usually, the chromatography is a method for separating a mixture according to its components.

[0003]

Figure 8 is a diagram illustrating a conventional chromatography specimen which is used for measurement by the chromatography.

In figure 8, a chromatography specimen 100 has a reactive layer carrier support body 101 which supports a chromatography

material, a sample application part 102 to which a liquid sample is applied, a marker hold region 103 which holds a marker reagent which can be moved by permeation of the liquid sample, a reactive layer 104 in which a binding reaction is processed between the marker reagent having a substance that is specifically bound to an analysis target included in a liquid sample which flows therein and the analysis target, a specific protein immobilization part 105 in which a specific protein that specifically processes a binding reaction with an analysis target such as an antibody and an antigen according to a reaction format is immobilized on the region of the reactive layer 104, and a water-absorbing part 106 for absorbing the liquid sample which flows therein.

[0004]

Next, a measuring method using the conventional chromatography specimen 100 will be described.

When a liquid sample is applied to the sample application part 102, the liquid sample permeates the sample application part 102 and reaches to the marker hold region 103. Then, a marker reagent held in the area of the marker hold region 103 is dissolved due to the permeation of the liquid sample and permeates the reactive layer 104 with the liquid sample. On the region of the reactive layer 104, there is the specific protein immobilization part 105 in which a specific protein is immobilized. When the liquid sample includes an analysis

target, the specific protein processes an antigen-antibody reaction with a complex of the analysis target and the marker reagent, and some color reaction is seen in the region of the specific protein immobilization part 105. When the liquid sample does not include an analysis target, no antigen-antibody reaction is processed nor no color reaction is seen. The liquid sample is finally absorbed into the water-absorbing part 106, and the reaction is ended. This color reaction is measured visually or by adopting a detection device. As described above, since the measuring method using chromatography can judge the test result very easily, it is widely applicable.

[0005]

The measurement using chromatography can be utilized for tests of various analysis targets. However, since a liquid sample is added, more uniform spread pattern of the liquid sample is required. Further, since the spread speed affects a reaction on the reactive layer 104, an improvement in the permeation on the reactive layer 104 is also required.

[0006]

Japanese Published Patent Application No. Sho.62-71861 discloses a method by which a surface active agent having a HLB value larger than 20 is applied during measurement employing immunity principles to improve the permeation on the reactive layer of the chromatography specimen. Further, Japanese

Published Patent Application No. Hei.11-153601 discloses a method in which an additive impregnating part which carries a surface active agent or the like is provided between the sample application part and the reactive layer.

[0007]

[Problems to be solved by the Invention]

For the conventional chromatography specimen, there is no means for mechanically controlling the permeation speed of a liquid sample, and thus it is impossible to artificially control the permeation speed, whereby the permeation speed of the liquid sample depends on the permeability of the specimen. Therefore, a considerable time may be required for permeation, permeation of the liquid sample to the reactive layer 104 may not be uniform, or a reactive component immobilization part such as the specific protein immobilization part 105 may have a part which is not permeated by the sample, thereby lacking precision in reaction.

[0008]

To solve the above-mentioned problems, conventionally, a surface active agent processing is executed to enhance the permeation performance of the chromatography specimen. However, when a surface active agent whose original property is in liquid or paste form is used, since it is impossible to dry the surface active agent up to an absolute dry condition, an immobilized antibody is gradually devitalized during a

conservation period of the chromatography specimen and the performance of the specimen is deteriorated. Thereby, a quality maintenance period of the specimen is shortened or a storage condition of the specimen is restricted.

[0009]

Further, in the measurement using chromatography, measurement is carried out when a predetermined period of time has passed after application of a liquid sample on a specimen. Therefore, when a surface active agent processing is carried out, a nonspecific adsorption of a marker reagent to the reactive layer occurs and thereby the marker reagent may remain on the reactive layer as a background. When the value of the background is added to the degree of coloring, an error thereof is undesirably included when measurement is carried out using a detecting instrument, resulting in a reduction in the quantitative performance. Also when judgement is carried out visually, the color of the background leads to an erroneous recognition in judgement of the essential color situation.

[0010]

Further, in the measurement method disclosed in Japanese Published Patent Application No. Sho.62-71861, since a surface active agent having a HLB value higher than 20 is applied to increase the permeability, the permeability and permeation speed of the specimen are surely increased, while the permeation speed is too high to perform a sufficient reaction

required for the measurement, resulting in the lack of precision in measurement.

[0011]

Further, in the chromatography specimen disclosed in the Japanese Published Patent Application No. Hei.11-153601, a surface active agent is impregnated on a region situated forward the reactive layer on the specimen to reduce influences of the background. In the case of using this chromatography specimen, since no surface active agent exists in the reactive component immobilization part such as a specific protein immobilization part, the reactive components are not devitalized nor denatured. However, since the sample does not sufficiently permeate the reactive components in the immobilization area in the stage where the permeation is promoted, the reaction is not performed uniformly, resulting in the lack of precision in measurement.

[0012]

The present invention is made to solve the above-mentioned problems and has for its object to provide a chromatography specimen and a manufacturing method thereof, which minimize the quantity of a marker reagent remaining in the background, enhance the reactivity by improvement of the permeability of a liquid sample and more uniform spread of the liquid sample, and enhance the preservation stability of the chromatography specimen.

[0013]

[Measures to solve the Problems]

In order to achieve the above-mentioned objects, a chromatography specimen according to Claim 1 is a chromatography specimen which is obtained by laminating plural porous materials or made of a single-layer porous material, wherein a reactive layer on which at least one of reactive components adopted in a chromatographic analysis is immobilized includes a surface active agent having such a property that it can be solidified when dried.

[0014]

Further, according to Claim 2, in the chromatography specimen defined in Claim 1, the surface active agent includes a surface active agent having a HLB value which is 20 or lower.

[0015]

Further, according to Claim 3, in the chromatography specimen defined in Claim 1 or 2, the surface active agent includes a nonionic surface active agent.

[0016]

Further, according to Claim 4, in the chromatography specimen defined in any of Claims 1 to 3, the surface active agent includes a cholic acid surface active agent.

[0017]

Further, according to Claim 5, in the chromatography specimen defined in any of Claims 1 to 4, the surface active

agent includes a surface active agent having sugar in a hydrophilic part.

[0018]

Further, according to Claim 6, in the chromatography specimen defined in any of Claims 1 to 5, the reactive layer includes the surface active agent in the entirety thereof.

[0019]

Further, according to Claim 7, in the chromatography specimen defined in any of Claims 1 to 5, the reactive layer includes the surface active agent in a part thereof.

[0020]

Further, a method for manufacturing a chromatography specimen according to Claim 8 is a method for manufacturing a chromatography specimen having a reactive layer on which at least one of reactive components adopted in a chromatographic analysis is immobilized, and the method comprises: a step of impregnating or coating the reactive layer of the chromatography specimen with a surface active agent dissolved liquid in which a surface active agent having such a property that it can be solidified when dried is dissolved; and a step of drying the surface active agent dissolved liquid with which the reactive layer has been impregnated or coated.

[0021]

Further, according to Claim 9, in the chromatography specimen manufacturing method defined in Claim 8, the surface

active agent includes a surface active agent having a HLB value which is 20 or lower.

[0022]

Further, according to Claim 10, in the chromatography specimen manufacturing method as defined in Claim 8 or 9, wherein the surface active agent includes a nonionic surface active agent.

[0023]

Further, according to Claim 11, in the chromatography specimen manufacturing method defined in any of Claims 8 to 10, the surface active agent includes a cholic acid surface active agent.

[0024]

Further, according to Claim 12, in the chromatography specimen manufacturing method defined in any of Claims 8 to 11, the surface active agent includes a surface active agent having sugar in a hydrophilic part.

[0025]

Further, according to Claim 13, in the chromatography specimen manufacturing method defined in any of Claims 8 to 12, the reactive layer is dried by air drying.

[0026]

Further, according to Claim 14, in the chromatography specimen manufacturing method defined in any of Claims 8 to 12, the reactive layer is dried by wind drying.

[0027]

Further, according to Claim 15, in the chromatography specimen manufacturing method defined in any of Claims 8 to 12, the reactive layer is dried by freeze drying.

[0028]

Further, according to Claim 16, in the chromatography specimen manufacturing method defined in any of Claims 8 to 15, the entire reactive layer is impregnated or coated with the surface active agent dissolved liquid.

[0029]

Further, according to Claim 17, in the chromatography specimen manufacturing method defined in any of Claims 8 to 15, a part of the reactive layer is impregnated or coated with the surface active agent dissolved liquid.

[0030]

[Embodiments of the Invention]

Hereinafter, embodiments according to the present invention will be described with reference to the drawings. The embodiments described here are given only as examples and the present invention is not restricted to these embodiments.

[0031]

(Embodiment 1)

Figure 1 is a diagram illustrating a lateral flow-type chromatography specimen made of a single-layer porous material according to a first embodiment of the present invention.

In figure 1, a lateral flow-type chromatography specimen 10 has a reactive layer carrier support 1, a sample application part 2, a marker hold region 3, a reactive layer 4, a specific protein immobilization part 5, and a water-absorbing part 6.

[0032]

The reactive layer carrier support 1 is made of liquid-impermeable plastic or the like, and supports a chromatography material. The sample application part 2 is made of a nonwoven fabric having high hydrophilia or the like, and a liquid sample is added or applied thereto. The marker hold region 3 holds a marker reagent so that it can be dissolved into the nonwoven fabric or the like. The reactive layer 4 is made of nitrocellulose or the like, and further impregnated with a surface active agent dissolved liquid in which a surface active agent having such a property that it can be solidified when dried is dissolved and thereafter dried. The surface active agent shown here having such a property that it can be solidified when dried is the one which can be massed or have a solid shape such as granules and powder when the surface active agent in a high concentration is subjected to a vacuum or freeze drying or to a drying operation at the normal pressures with the addition of heat or at the ordinary temperatures and normal pressures. Further, the impregnation process to the reactive layer 4 is a process for dipping the reactive layer 4 in the surface active agent dissolved liquid. The specific

protein immobilization part 5 is obtained by immobilization a specific protein that specifically processes a binding reaction with an analysis target like an antibody or an antigen according to the reaction format on the region of the reactive layer 4. The water-absorbing part 6 finally absorbs the liquid sample. The sample application part 2, the marker hold region 3, the reactive layer 4, the specific protein immobilization part 5, and the water-absorbing part 6 are formed on the top of the reactive layer carrier support 1. The marker reagent and the specific protein should be selected properly according to a sample to be analyzed and an analysis target.

[0033]

As the surface active agent to be used for the surface active agent dissolved liquid, for example, a surface active agent having a HLB value of 20 or lower, a nonionic surface active agent, a cholic acid surface active agent, or a surface active agent having sugar in a hydrophilic part may be employed.

[0034]

As the processing for drying the surface active agent dissolved liquid with which the reactive layer 4 has been impregnated, for example, there are processings such as air drying which is a method of leaving the liquid under room temperature and normal pressure to dry it, wind drying which is a method of applying a prescribed wind force to the liquid at an arbitrary temperature to dry it, and freeze drying.

[0035]

Further, as the lateral flow-type chromatography specimen 10, a specimen comprising a chromatography material which is composed of an arbitrary porous carrier such as nitrocellulose or glass fiber filter paper is employed.

[0036]

Next, a chromatographic analysis method adopting the lateral flow-type chromatography specimen 10 according to the first embodiment will be described.

[0037]

In figure 1, when a liquid sample is applied to the sample application part 2, the liquid sample permeates the sample application part 2 and reaches to the marker hold region 3. Then, a marker reagent held in the area of the marker hold region 3 is dissolved due to the permeation of the liquid sample and permeates the reactive layer 4 together with the liquid sample. Then, a surface active agent included in the reactive layer 4 is dissolved with the permeation of the liquid sample. By the action of the dissolved surface active agent, permeation into the reactive layer 4 is speedily performed and the permeation of the liquid sample is proceeded with the end of the permeating liquid being lined and without remaining. In the area of the reactive layer 4, there is the specific protein immobilization part 5 on which a specific protein is immobilized. When the liquid sample includes an analysis

target, the specific protein processes an antigen-antibody reaction with a complex of the analysis target and the marker reagent, resulting in some color reaction in the area of the specific protein immobilization part 5. When the liquid sample does not include an analysis target, the antigen-antibody reaction is not processed and no color reaction is seen. Finally, the liquid sample is absorbed by the water-absorbing part 6 and the reaction is ended.

[0038]

As described above, according to the lateral flow-type chromatography specimen 10 of the first embodiment, the permeability of the reactive layer 4 is enhanced and more uniform permeation is carried out by the impregnation processing of the surface active agent dissolved liquid on the chromatography specimen 10 and the drying processing. This enhancement in permeability and the uniform permeation improve the reactivity of the chromatography specimen 10, resulting in a chromatography measurement with a higher sensitivity and a higher performance. Further, since the surface active agent having such a property that it can be solidified when dried is employed, the reactive layer 4 is in a completely dried condition until the liquid sample is applied thereto and permeates the reactive layer 4. Therefore, devitalization of the immobilized specific protein can be minimized, resulting in enhancement in preservation stability, extension of the quality

maintenance period, and relaxation of storage conditions on the chromatography specimen 10.

[0039]

In addition, when the surface active agent comprising the surface active agent which has a HLB value of 20 or lower is adopted, it can be avoided that the permeation speed of the liquid sample into the reactive layer 4 becomes too high to obtain a sufficient reaction. Further, the reaction speed can be appropriately adjusted by selecting the HLB value, whereby a chromatography measurement with a higher sensitivity and a higher performance can be realized.

[0040]

When the surface active agent comprising a nonionic surface active agent is adopted, nonspecific adsorption of the marker reagent onto the reactive layer 4 is avoided, and the marker reagent is prevented from remaining on the reactive layer 4 as the background, resulting in measurement with the chromatography specimen having a higher sensitivity and a higher performance.

[0041]

Further, when the surface active agent comprising the cholic acid surface active agent is adopted, influence upon protein can be reduced, and denaturation or devitalization of the immobilized specific protein can be minimized, whereby the performance of the reactive layer 4 can be held for a long time.

[0042]

Further, when the surface active agent comprising the surface active agent which has sugar in its hydrophilic part is adopted, the solubility is increased and the permeability is enhanced by the action of sugar while the denaturation or devitalization of the immobilized specific protein can be minimized because the influence upon protein is insignificant, whereby the performance of the reactive layer 4 can be held for a long time.

[0043]

Further, when the reactive layer is dried by air drying, the load onto the specific protein immobilized on the reactive layer can be suppressed, whereby the performance of the specimen can be held for a long time.

Furthermore, when the reactive layer is dried by wind drying, the drying time can be shortened, and devitalization or denaturation of the specific protein during the drying can be minimized, whereby the performance of the specimen can be held for a long time.

Moreover, when the reactive layer is dried by freeze drying, the properties of the specific protein can be almost held, whereby the performance of the specimen can be held for a long time.

[0044]

(Embodiment 2)

Hereinafter, a flow through-type chromatography specimen according to a second embodiment of the present invention will be described with reference to figures 2, 3 and 4.

[0045]

Figure 2 is a perspective view illustrating a structure of a flow through-type chromatography specimen which is constituted by laminating plural porous materials according to the second embodiment. Figure 3 is a perspective view illustrating the flow through-type chromatography specimen seen from the side of a sample application part. Figure 4 is a perspective view of the flow through-type chromatography specimen seen from the side of a water-absorbing part for finally absorbing a liquid sample.

[0046]

In figure 2, the flow through-type chromatography specimen 20 has a sample application part 11, a marker hold region 12, a specific protein immobilization part 13, a reactive layer 14, and a water-absorbing part 15.

The sample application part 11 is made of a nonwoven fabric having a high hydrophilia or the like, and a liquid sample is added or applied thereto. The marker hold region 12 holds a marker reagent in the nonwoven fabric or the like so that it can be dissolved. The specific protein immobilization part 13 is obtained by immobilizing a specific protein that specifically performs a binding reaction with an analysis

target such as an antibody or antigen according to the reaction format on the area of the reactive layer 14. The reactive layer 4 is made of nitrocellulose or the like, and it is subjected to impregnation processing with a surface active agent dissolved liquid in which a surface active agent having such a property that it can be solidified when dried is dissolved, and thereafter, is dried. The impregnation processing performed on the reactive layer 14 is a processing of soaking the reactive layer 14 in the surface active agent dissolved liquid. The water-absorbing part 15 has a result confirmation window 16 for seeing the result of the reaction on the reactive layer 14 and finally absorbs the liquid sample. The sample application part 11, the marker hold region 12, the specific protein immobilization part 13, the reactive layer 14, and the water-absorbing part 15 are laminated, thereby forming the flow through-type chromatography specimen 20. The marker reagent and the specific protein should be selected appropriately according to a sample to be analyzed and an analysis target.

[0047]

As the surface active agent to be used for the surface active agent dissolved liquid, for example, a surface active agent having a HLB value of 20 or lower, a nonionic surface active agent, a cholic acid surface active agent, or a surface active agent having sugar in a hydrophilic part may be employed.

Further, as the processing for drying the surface active agent dissolved liquid with which the reactive layer 4 has been impregnated, for example, there are processings such as air drying which is a method of leaving the liquid under room temperature and normal pressure to dry it, wind drying which is a method of applying a prescribed wind force to the liquid at an arbitrary temperature to dry it, and freeze drying.

Furthermore, as the lateral flow-type chromatography specimen 20, a specimen comprising a chromatography material which is composed of an arbitrary porous carrier such as nitrocellulose or glass fiber filter paper is employed.

[0048]

Next, a chromatographic analysis method which adopts the flow through-type chromatography specimen 20 according to the second embodiment will be described.

On the flow through-type chromatography specimen 20 shown in figures 2, 3 and 4, when a liquid sample is applied to the sample application part 11, the liquid sample permeates the sample application part 11 and reaches to the marker hold region 12. Then, a marker reagent held in the area of the marker hold region 12 is dissolved due to the permeation of the liquid sample and permeates the reactive layer 14 together with the liquid sample. Then, a surface active agent included in the reactive layer 14 is dissolved with the permeation of the liquid sample. By the action of the dissolved surface active

agent, permeation into the reactive layer 14 is speedily performed and the permeation of the liquid sample is proceeded with the end of the permeating liquid being relatively uniformed and without remaining. On the area of the reactive layer 14, there is the specific protein immobilization part 13 on which a specific protein is immobilized. When the liquid sample includes an analysis target, the specific protein processes an antigen-antibody reaction with a complex of the analysis target and the marker reagent, resulting in some color reaction in the area of the specific protein immobilization part 13. The color reaction can be seen through the result confirmation window 16 provided in the water-absorbing part 15. When the liquid sample does not include an analysis target, the antigen-antibody reaction is not processed and no color reaction is shown. The liquid sample is finally absorbed into the water-absorbing part 15, and then the reaction is ended.

[0049]

As described above, according to the flow through-type chromatography specimen 20 of the second embodiment, the permeability of the reactive layer 14 is enhanced and a more uniform permeation is enabled by the impregnation processing and the drying processing of the surface active agent dissolved liquid on the flow through-type chromatography specimen 20. The enhancement in the permeability and the uniform permeation improve the reactivity of the chromatography specimen 20,

resulting in a chromatography measurement with a higher sensitivity and a higher performance. Further, since the surface active agent having such a property that it can be solidified when dried is employed, the reactive layer 14 is in a completely dried condition until the liquid sample is applied thereto and permeates the reactive layer 14. Therefore, devitalization of the immobilized specific protein can be minimized, resulting in enhancement in preservation stability, extension of the quality maintenance period, and relaxation of storage conditions on the chromatography specimen.

[0050]

In addition, by employing, as a surface active agent, one including a surface active agent which has a HLB value of 20 or lower, it can be avoided that the permeation speed of the liquid sample into the reactive layer 14 becomes too high to obtain a sufficient reaction. Further, the reaction speed can be appropriately adjusted by selecting the HLB value, whereby a chromatography measurement with a higher sensitivity and a higher performance can be realized.

[0051]

Further, by employing, as a surface active agent, one including a nonionic surface active agent, nonspecific adsorption of the marker reagent onto the reactive layer 14 is avoided, and the marker reagent is prevented from remaining on the reactive layer 14 as the background, resulting in

measurement with the chromatography specimen having a higher sensitivity and a higher performance.

[0052]

Further, by employing, as a surface active agent, one including a cholic acid surface active agent, influence upon protein can be reduced, and denaturation or devitalization of the immobilized specific protein can be minimized, whereby the performance of the reactive layer 14 can be held for a long time.

[0053]

Further, by employing, as a surface active agent, one including a surface active agent which has sugar in its hydrophilic part, the solubility is increased and the permeability is enhanced by the action of sugar while the denaturation or devitalization of the immobilized specific protein can be minimized because the influence upon protein is insignificant, whereby the performance of the reactive layer 14 can be held for a long time.

[0054]

Further, when the reactive layer is dried by air drying, the load onto the specific protein immobilized on the reactive layer can be suppressed, whereby the performance of the specimen can be held for a long time.

Furthermore, when the reactive layer is dried by wind drying, the drying time can be shortened, and devitalization or

denaturation of the specific protein during the drying can be minimized, whereby the performance of the specimen can be held for a long time.

Moreover, when the reactive layer is dried by freeze drying, the properties of the specific protein can be almost held, whereby the performance of the specimen can be held for a long time.

[0055]

The surface active agent employed in the first and second embodiments is the general term for substances which include a hydrophilic atomic group having a high affinity with water molecules and a hydrophobic atomic group having a low affinity with water molecules in its molecule, and have properties of changing properties on the interface or surface.

[0056]

While in the first and second embodiments a description has been given of the specimen in which the specific protein is immobilized on the reactive layer as the reactive component to be used for the chromatographic analysis, this is merely an example. For example, a reactive component such as an enzyme, which causes some changes before and after the reaction, may be adopted as the reactive component to be immobilized onto the reactive layer.

[0057]

Figures 5 and 6 are diagrams illustrating a structure of

a chromatography specimen that adopts an enzyme.

A chromatography specimen 30 has a sample application part 11, a reactive reagent impregnation region 17, a reactive layer 14, an enzyme immobilization part 7, and a water-absorbing part 15. Since the constituents other than the enzyme immobilization part 7 obtained by immobilizing an enzyme onto an area of the reactive layer 14 are identical to those of the second embodiment, description thereof will be omitted.

[0058]

In the measurement with the chromatography specimen 30 using an enzyme, when the liquid sample includes an analysis target, some color reaction is seen in the area of the enzyme immobilization part 7 by the action of the reactive reagent with which the reactive reagent impregnation region 17 is impregnated and the enzyme immobilized on the enzyme immobilization part 7.

[0059]

Also in the chromatography specimen 30, the reactive layer 14 is impregnated with the surface active agent dissolved liquid and then dried, whereby the same effects as those described for the second embodiment can be achieved.

[0060]

While the first and second embodiments employ, as a surface active agent, one including a surface active agent having a HLB value of 20 or lower, these embodiments may

preferably employ one including a surface active agent that has a HLB value near 20 and has a structure in which many hydrophilic atomic groups are included.

[0061]

Further, while the first and second embodiments employ, as a surface active agent, one including a cholic acid surface active agent, these embodiments may preferably employ one including a surface active agent having a cholic acid such as N, N-Bis (3-D-gluconamidopropyl) cholamide or N, N-Bis (3-D-gluconamidopropyl) deoxycholamide as its mother nucleus.

[0062]

Further, while the first and second embodiments employ one including a surface active agent having sugar in its hydrophilic part, these embodiments may preferably employ one including a surface active agent of a structure having a sugar chain such as Sucrose Monolaurate or n-Octyl-ß-D-Thioglucoside.

[0063]

Further, in the first and second embodiments, the surface active agent processing to the reactive layer 4 or 14 may be performed to a part or the whole of the reactive layer 4 or 14.

[0064]

Further, while in the first and second embodiments the surface active agent processing to the reactive layer 4 or 14 is carried out by the impregnation processing which impregnates the reactive layer 4 or 14 with the surface active agent

dissolved liquid, it may be performed by a coating processing which coats the reactive layer 4 or 14 with the surface active agent dissolved liquid.

[0065]

Furthermore, the above-described material names of the surface active agents are merely examples, and a surface active agent other than those described above may be employed.

[0066]

[Examples]

A method for implementing the present invention will be described in more detail through the following examples. The present invention is not restricted by the following examples.

[0067]

(Quantitative analysis of hCG in blood plasma)

A lateral flow-type immunochromatography specimen which includes an anti-hCG-ß antibody immobilization line and a broad band of a complex of an anti-hCG-α antibody and gold colloid in a nitrocellulose film is manufactured. This specimen is shown in figure 1. In this figure, the specimen includes a specific protein immobilization part 5, a marker hold region 3 located forward the part 5, which is an area including a complex of an anti-hCG-α antibody and a gold colloid, and a sample application part 2. The specimen is manufactured as follows.

[0068]

Example 1.

Preparation of chromatography specimen

An anti-hCG-ß antibody solution which was diluted with a phosphate buffer solution to control the concentration was prepared. This antibody solution was applied on the nitrocellulose film by adopting a solution discharge device. Thereby, a detecting antibody immobilization line was obtained on the nitrocellulose film. After being dried, this nitrocellulose film was immersed in a Tris-HCl buffer solution including 1% skim milk and shaken gently for 30 minutes. 30 minutes later, the nitrocellulose film was moved into a Tris-HCl buffer solution tank, shaken gently for 10 minutes, and thereafter shaken gently in another Tris-HCl buffer solution tank for another 10 minutes, to wash the nitrocellulose film. After washed twice, the nitrocellulose film was immersed in a Tris-HCl buffer solution including 0.05% Sucrose Monolaurate (Dojindo made), shaken for 10 minutes, then taken out from the solution tank, and dried at room temperature.

[0069]

The gold colloid was prepared by adding 1% citric acid solution to a refluxing 100°C-solution of 0.01% chloroauric acid. After the reflux was continued for 30 minutes, it was cooled. The anti-hCG-α antibody was added to gold colloid solution which was prepared to pH9 by using 0.2M potassium carbonate solution, then the obtained solution was stirred for several minutes, and then 10% BSA (bovine serum albumin)

solution pH9 was added thereto by such an amount that 1% solution was finally obtained and stirred. Thereby, an antibody-gold colloid complex (marker antibody) was prepared. The marker antibody solution was centrifuged at 4°C and 20000G for 50 minutes, whereby the marker antibody was isolated, and the isolated marker antibody was suspended in a washing buffer solution (1% BSA·phosphate buffer solution) and thereafter centrifuged to wash and isolate the marker antibody. After suspended in the washing buffer solution and filtrated through a 0.8μm filter, the marker antibody was prepared one-tenth as much as the initial gold colloid solution and stored at 4°C.

[0070]

The marker antibody solution was set in the solution discharge device and applied to a position on an anti-hCG-ß antibody immobilization dry film, apart from an antibody immobilization position, and thereafter the film was dried. Thereby, the marker antibody hold region was obtained on the immobilization film.

[0071]

The antibody immobilization film including the marker antibody hold region 3 prepared as described above was affixed on the reactive layer carrier support, a nonwoven fabric was added thereto as the sample application part, glass fiber filter paper was added thereto as the water-absorbing part, and thereafter, the film was cut into small pieces of 0.5cm width,

thereby manufacturing the specimen.

[0072]

Example 2.

Preparation of sample

Human blood to which heparin was added as an anticoagulant was centrifuged at 4000 rpm for 5 minutes to prepare blood plasma in which blood cells were separated. The hCG solutions of known concentrations were added to the plasma, thereby preparing the hCG solutions of various known concentrations.

[0073]

Example 3.

Measurement of the degree of coloration on specimen

More than 200μl of plasma including hCG was applied to the sample application part on the specimen and spread in the direction of the water-absorbing part, to make an antigen-antibody reaction, whereby a color reaction in the antibody immobilization part is carried out. Then, the coloration state 5 minutes after the sample application to the specimen was measured by adopting a reflective spectrophotometer (CS9300; Shimadzu Corporation made), and the coloration degree is computed.

[0074]

Then, plasmas including hCG of 0, 100, 1000, and 10000U/1 were applied to the specimen to be spread, and the coloration state of the antibody immobilization part on the specimen for

plasma of each hCG concentration was measured by a reflective spectrophotometer. An absorbance at the wavelength of 520nm was measured, and substituted into a previously formed calibration curve indicating a relationship between the hCG concentration and the absorbance. The result is shown in figure 7. Essentially, when, for example, the absorbance of blood including hCG of 1000U/1 was measured and the measured absorbance was substituted into the calibration curve, the hCG concentration should be all 1000U/1. However, there is actually a little deviation. According to the degree of the deviation, accuracy of the measurement can be known.

[0075]

Figures 7(a) and 7(b) are diagrams illustrating quantitative performances in the chromatography specimen, figure 7(a) showing a case where the reactive layer is not processed with a surface active agent while figure 7(b) showing a case where it is processed with the surface active agent. The abscissa represents the hCG concentration of a sample applied to the specimen 10. The ordinate represents the converted value of the antigen concentration obtained by substituting a signal from a marker in the color area on the specimen into the calibration curve.

[0076]

Hereinafter, descriptions will be given of effects in a case where the reactive layer is not processed with a surface

active agent and a case where it has been processed with the surface active agent in the chromatography specimen.

Figure 7 shows the result obtained by converting the concentration of an analysis target on the basis of a measured value of a coloration degree, five minutes after a liquid sample is applied to the chromatography specimen. The marker reagent used at this time is the same antibody-gold colloid complex both in figures 7(a) and 7(b). In a case where the reactive layer has been processed with a surface active agent (figure 7(b)), a CV value (coefficient of variation) is within 0 to 7%, while in a case where it is not processed with a surface active agent (figure 7(a)), the CV value ranges from 15 to 35% having a wide range of variations, and has a low quantitative performance. From the above results, it can be understood that the use of the reactive layer which has been impregnated with the surface active agent in the chromatography specimen greatly concerns the enhancement in the quantitative performance. While in this example the comparison description has been given with respect to the quantitative performance, a more accurate result is obtained also in a qualitative test when a chromatography specimen having a reactive layer which is impregnated with a surface active agent is adopted.

[0077]

Since the gold colloid is adopted as the marker in this example, the coloration degree at a wavelength of 520nm is

measured. However, an absorbance at any wavelength may be measured as long as it is an absorption wavelength of the marker.

[0078]

[Effects of the Invention]

According to Claims 1, 6, and 7, in a chromatography specimen which is obtained by laminating plural wettable porous materials or made of a single-layer porous material, a reactive layer on which at least one of reactive components adopted in a chromatographic analysis is immobilized includes a surface active agent having such a property that it can be solidified when dried. Therefore, the reactivity of the chromatography specimen can be enhanced by the enhancement in the permeability of the reactive layer and the uniform permeation of a sample, thereby realizing a chromatography measurement with a higher sensitivity and a higher performance. Further, by adopting, as the surface active agent, a surface active agent having such a property that it can be solidified when dried, devitalization of reactive components immobilized on the reactive layer can be minimized, thereby realizing enhanced preservation stability, extended quality maintenance period, and relaxed storage condition of the chromatography specimen.

[0079]

According to a chromatography specimen defined in Claim 2, the surface active agent includes a surface active agent having

a HLB value of 20 or lower. Therefore, in addition to the effects of Claim 1, it can be prevented that the permeation speed of a liquid reagent in the reactive layer is too high to obtain a sufficient reaction, and further, the reaction speed can be appropriately controlled by selecting the HLB value, resulting in a chromatography measurement with a higher sensitivity and a higher performance.

[0080]

According to a chromatography specimen defined in Claim 3, the surface active agent includes a nonionic surface active agent. Therefore, in addition to the effects of Claim 1, a nonspecific adsorption of a marker reagent onto the reactive layer is avoided, and it can be prevented that the marker reagent remains on the reactive layer as a background, thereby realizing a measurement by a chromatography specimen with a higher sensitivity and a higher performance.

[0081]

According to a chromatography specimen defined in Claim 4, the surface active agent includes a cholic acid surface active agent. Therefore, in addition to the effects of Claim 1, influence upon protein can be reduced and denaturation or devitalization of immobilized specific protein can be minimized, whereby the performance of the reactive layer can be held for a long time.

[0082]

According to a chromatography specimen defined in Claim 5, the surface active agent includes a surface active agent having sugar in a hydrophilic part. Therefore, in addition to the effects of Claim 1, the solubility is enhanced and the permeability is increased by the action of sugar, while influence upon protein can be reduced, whereby denaturation or devitalization of immobilized specific protein can be minimized and thus the performance of the reactive layer can be held for a long time.

[0083]

According to Claims 8, 16, and 17, a method for manufacturing a chromatography specimen which has a reactive layer on which at least one of reactive components adopted in a chromatographic analysis is immobilized, comprises a step of impregnating or coating the reactive layer of the chromatography specimen with a surface active agent dissolved liquid in which a surface active agent having such a property that it can be solidified when dried is dissolved; and a step of drying the surface active agent dissolved liquid with which the reactive layer has been impregnated or coated. Therefore, the reactivity of the chromatography specimen can be enhanced due to enhancement in permeability of the reactive layer and uniform permeation of a sample, whereby a chromatography specimen with a higher sensitivity and a higher performance can be manufactured. Further, by adopting, as the surface active

agent, a surface active agent having such a property that it can be solidified when dried, devitalization of reactive components immobilized on the reactive layer can be minimized, whereby a chromatography specimen having enhanced preservation stability, extended quality maintenance period, and relaxed storage condition can be manufactured.

[0084]

According to a chromatography specimen manufacturing method defined in Claim 9, the surface active agent comprises a surface active agent having a HLB value which is 20 or lower. Therefore, in addition to the effects of Claim 8, it can be prevented that the permeation speed of a liquid sample in the reactive layer is too high to obtain a sufficient reaction, and further, the reaction speed can be appropriately controlled by selecting the HLB value, whereby a chromatography specimen with a higher sensitivity and a higher performance can be manufactured.

[0085]

According to a chromatography specimen manufacturing method defined in Claim 10, the surface active agent comprises a nonionic surface active agent. Therefore, in addition to the effects of Claim 8, a nonspecific adsorption of a marker reagent onto the reactive layer is avoided, and it can be prevented that the marker reagent remains on the reactive layer as a background, whereby a chromatography specimen with a

higher sensitivity and a higher performance can be manufactured.

[0086]

According to a chromatography specimen manufacturing method defined in Claim 11, the surface active agent comprises a cholic acid surface active agent. Therefore, in addition to the effects of Claim 8, influence upon protein can be reduced and denaturation or devitalization of immobilized specific protein can be minimized, whereby a chromatography specimen whose performance can be held for a long time can be manufactured.

[0087]

According to a chromatography specimen manufacturing method defined in Claim 12, the surface active agent includes a surface active agent having sugar in a hydrophilic part. Therefore, in addition to the effects of Claim 8, the solubility is enhanced and the permeability is increased by the action of sugar, while influence upon protein can be reduced, whereby denaturation or devitalization of immobilized specific protein can be minimized and thus a chromatography specimen whose performance can be held for a long time can be manufactured.

[0088]

According to a chromatography specimen manufacturing method defined in Claim 13, the reactive layer is dried by air drying. Therefore, in addition to the effects of Claim 8, the

load to a reactive component immobilized on the reactive layer can be suppressed, thereby manufacturing a chromatography specimen in which the performance of the processed reactive layer can be held for a long time.

[0089]

According to a chromatography specimen manufacturing method defined in Claim 14, the reactive layer is dried by wind drying. Therefore, in addition to the effects of Claim 8, the drying time can be shorten and devitalization or denaturation of an immobilized reactive component during the drying can be minimized, thereby manufacturing a chromatography specimen in which the performance of the processed reactive layer can be held for a long time.

[0090]

According to a chromatography specimen manufacturing method defined in Claim 15, the reactive layer is dried by freeze drying. Therefore, in addition to the effects of Claim 8, the property of an immobilized reactive component can be almost held, thereby manufacturing a chromatography specimen in which the performance of the processed reactive layer can be held for a long time.

[Brief Description of the Drawings]

[Figure 1]

A diagram illustrating the structure of a lateral flow-type chromatography specimen according to the first embodiment

of the present invention.

[Figure 2]

A perspective view illustrating the structure of a flow through-type chromatography specimen according to the second embodiment of the present invention.

[Figure 3]

A perspective view illustrating the flow through-type chromatography specimen according to the second embodiment of the invention.

[Figure 4]

A perspective view illustrating the flow through-type chromatography specimen according to the second embodiment of the invention.

[Figure 5]

A diagram illustrating the structure of a chromatography specimen using an enzyme.

[Figure 6]

A diagram illustrating the structure of a chromatography specimen using an enzyme.

[Figure 7]

Diagrams illustrating quantitative performances according to an example of the present invention, figure 7(a) showing a case where a surface active agent is not used in a processing for a reactive layer while figure 7(b) showing a case where a surface active agent is used.

[Figure 8]

A diagram illustrating a conventional chromatography specimen.

[Description of Reference Numerals]

1 ... reactive layer carrier support body

2,11 ... sample application part

3,12 ... marker hold region

4,14 ... reactive layer

5,13 ... specific protein immobilization part

6,15 ... water-absorbing part

7 ... enzyme immobilization part

16 ... result confirmation window

17 ... reactive reagent impregnation region

10 ... lateral flow-type chromatography specimen

20 ... flow through-type chromatography specimen

30 ... chromatography specimen

[Name of the Document] Abstract

[Summary]

[Object] It is an object to provide a chromatography specimen and a manufacturing method thereof, which can realize improvements in permeation speed and permeability on a reactive layer of the chromatography specimen, uniform permeation, and improvement in measuring performance.

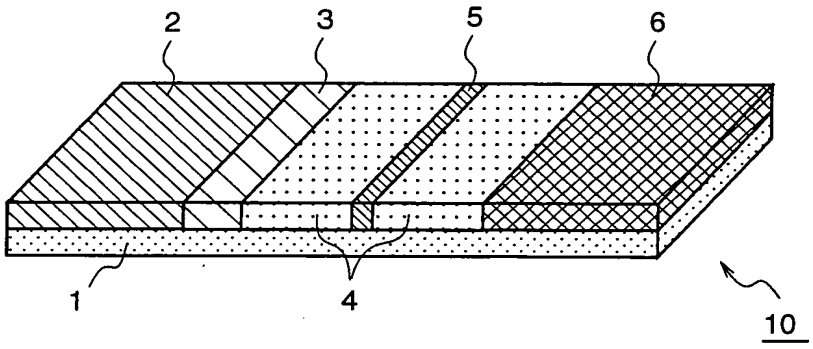
[Solution] A chromatography specimen 10 is constituted by a sample application part 2 to which a liquid sample is applied, a marker hold region 3 which holds a marker reagent, a reactive layer 4 where a binding reaction between the marker reagent and an analysis target is processed, a specific protein immobilization part 5 in which a specific protein is held in the area of the reactive layer 4, and a water-absorbing part 6 for absorbing a sample, and the reactive layer 4 is impregnated with a surface active agent dissolved solution having such a property that it can be solidified when dried, and thereafter, the reactive layer 4 is dried.

[Selected Figure] Figure 1

Name of Document

【書類名】　図　面　　Drawing

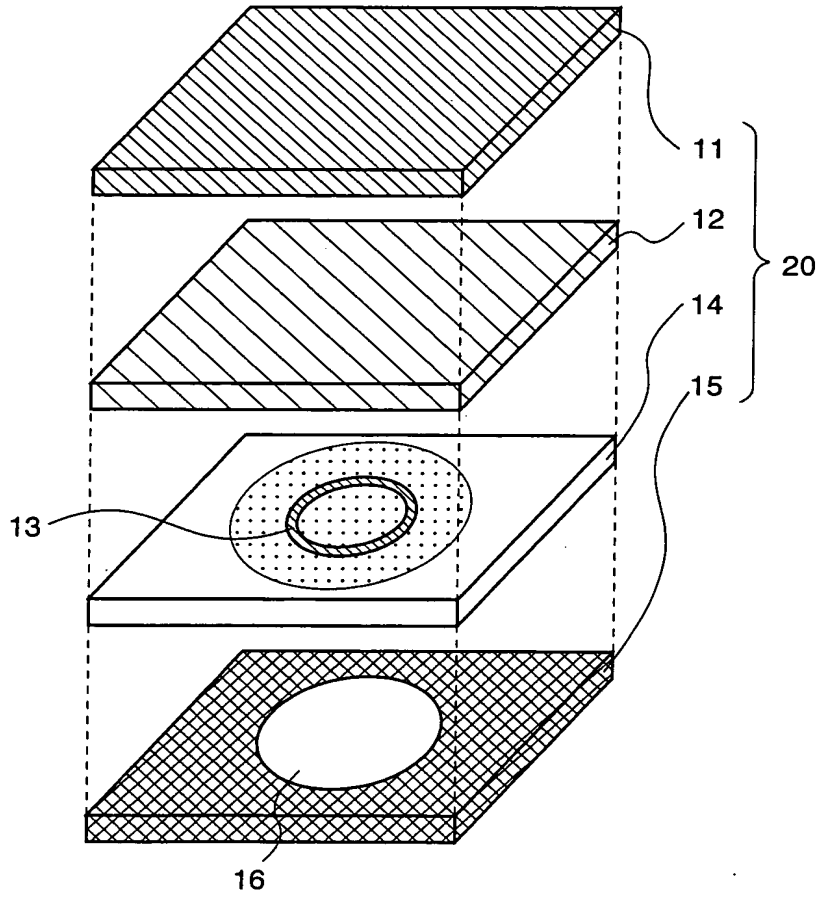
【図1】　Figure 1



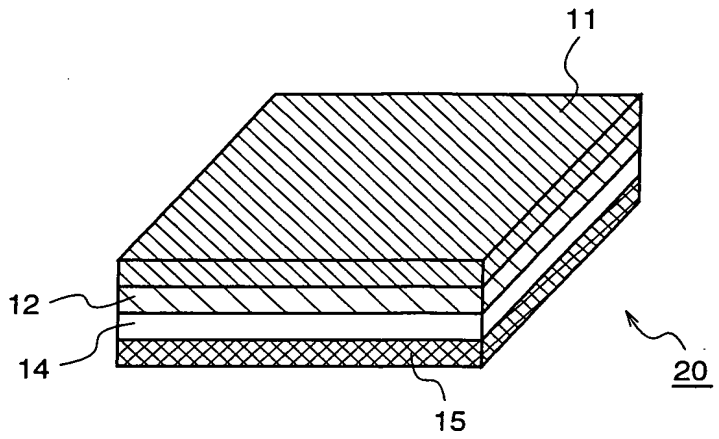
4: reactive layer
5: specific protein immobilization part
10: lateral flow-type chromatography specimen

4：反応層
5：特異的タンパク固定化部
10：模型クロマトグラフィー試験片

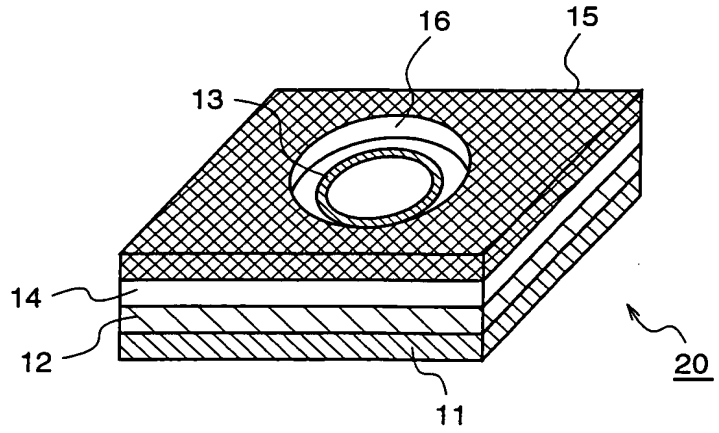
【図2】　Figure 2



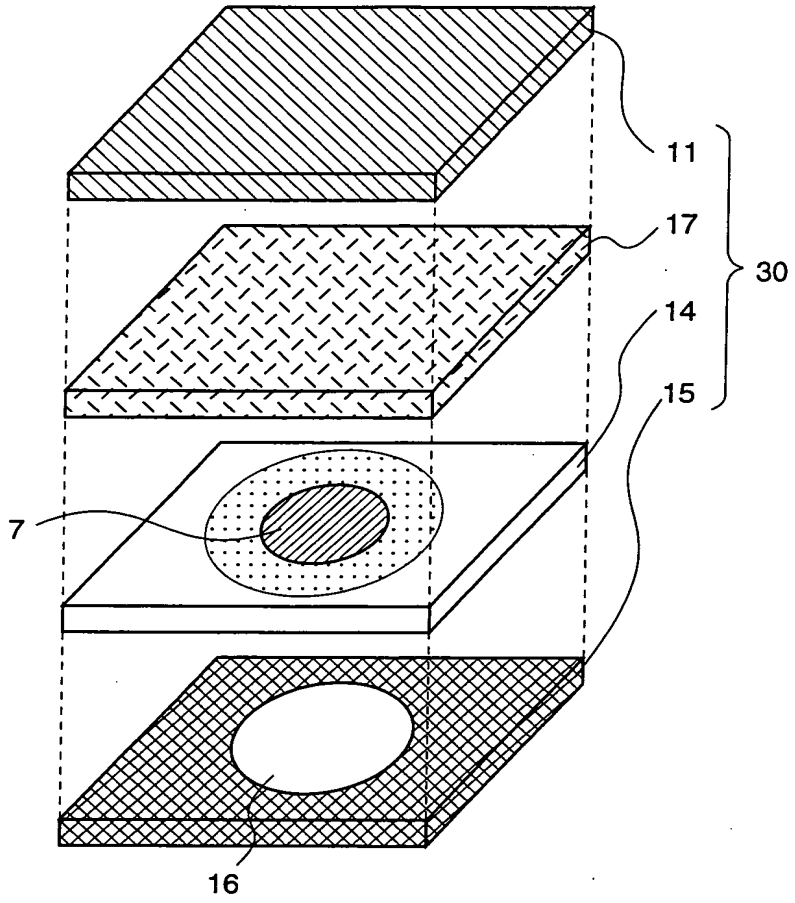
【図3】 Figure 3



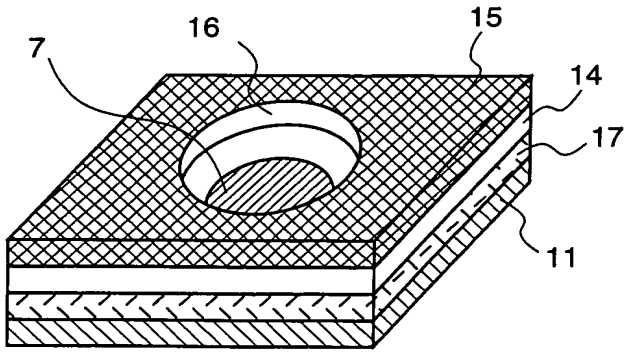
【図4】 Figure 4



【図5】 Figure 5

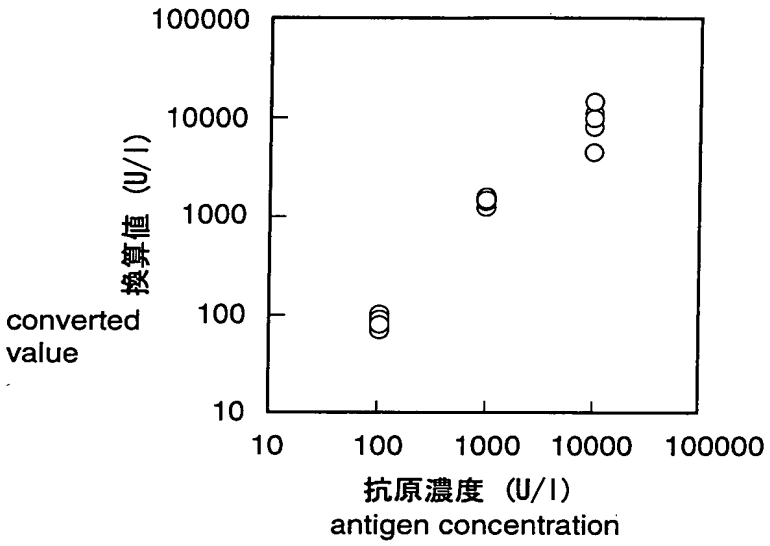


【図6】 Figure 6

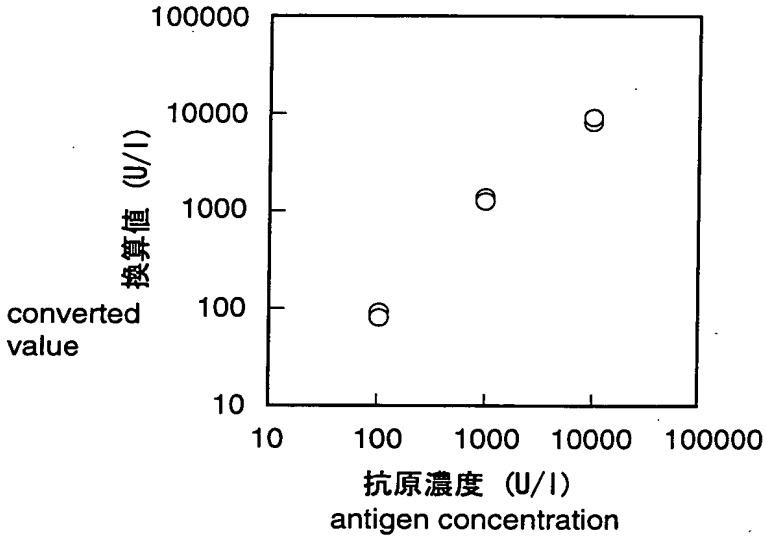


【図7】 Figure 7

(a) 界面活性剤なし without surface active agent



(b) 界面活性剤あり with surface active agent



【図8】 Figure 8